

BAY-OCEAN EXCHANGE PROCESSES: DEVELOPMENT AND APPLICATION OF A MEROPLANKTON TRACER TECHNIQUE

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Abstract

Further advancement in understanding the dynamics of larval exchange between estuarine and coastal marine benthic invertebrate populations requires (1) knowing the origins of field-sampled larvae and (2) synoptic assessments of horizontal and vertical larval distribution patterns over large areas for extended periods. Larval concentration and water velocity data were sampled concurrently and used to estimate larval flux rates between regions of San Diego Bay (SDB) and nearshore coastal waters in southern California. Simulations with a 2-D hydrodynamic model of SDB indicated widely differing larval transport probabilities depending on whether tidal vertical migration behavior occurs in the water column. Field studies indicate that crab (*Pachygrapsus crassipes*) zoeae migrate vertically in SDB, a behavior that promotes transport out of the Bay. In contrast, larvae of other crab species (*Lophopanopeus* spp.), which do not migrate vertically, are retained within SDB during development. An elemental larval fingerprinting technique (based on Cu, Zn, Al, Mn, and Sr) was developed to distinguish SDB from non-SDB spawned *P. crassipes* zoeae. With this method, bi-directional larval exchange was observed between SDB and coastal waters. Approximately 26% of *P. crassipes* larvae observed at the SDB entrance, and 5% at a mid-bay site, originated outside the Bay. This exchange is likely to have significant consequences for larval populations. Laboratory experiments revealed reduced survivorship in larva spawned from or reared in SDB water relative to pristine coastal waters. Combined use of trace elemental fingerprinting and synoptic field methods can facilitate understanding of larval transport and ultimately population dynamics of coastal species.

Subject Terms: San Diego Bay, larval dispersal, trace-elemental tagging,
bay-ocean exchange, vertical migration, crab larvae, zoeae

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Introduction

The early life history of most nearshore and estuarine benthic invertebrate species involves the release of a planktonic larval stage (i.e., meroplankton) which acts as an agent of dispersal and gene flow (Grahame and Branch 1985, Levin and Bridges 1995). A detailed understanding of dispersal trajectories and the magnitude of larval transport are fundamental to understanding the complexities and fate of larvae in the plankton, including the origin of recruits (Levin 1990) and sources and rates of mortality (Rumrill 1990, Morgan 1995).

In some populations, larval development occurs entirely in coastal waters or within embayments. For others, the life history may involve larval development in both open water and bay settings. Some species have larvae that are retained within estuaries throughout larval development until ready to settle and recruit to local adult populations (e.g., Cronin 1982). Other taxa preferentially export larvae to open coastal waters where they develop prior to reentering estuaries and recruiting to adult populations (e.g., Christy and Stancyk 1982). Mechanisms mediating the movements of larvae into and out of estuaries include passive transport (larval movement controlled by ambient physical processes) and active transport (larval behavior, such as vertical migration coupled with physical processes to allow differential horizontal advection) (Butman 1987, Young 1995, Shanks 1995). The ability of many of these larvae to exit or return to estuarine habitats has been considered critical for survival (Morgan 1995). This is the case for estuarine species that release planktonic larvae that develop offshore but recruit to adult populations located within estuaries (Epifanio 1988).

A number of marine invertebrate species inhabit both bay and exposed coastal environments. For these, failure to return to estuarine habitats may not be synonymous with death. Gaines and Bertness (1992) demonstrated that there was no significant correlation between interannual variation in settlement of the barnacle *Semibalanus balanoides* and variable reproductive output by adults or mortality rates during larval development. Instead, variability in settlement and recruitment was explained by interannual variations in estuarine flushing rates. Gaines and Bertness' (1992) study showed that for species which have adult populations inhabiting both bay and coastal settings, the exchange of larvae between these two environments is likely and may have significant consequences for population maintenance.

The overall objective of this research was to develop methods to track the exchange of planktonic larvae between San Diego Bay and open coast habitats. Brachyuran larvae, in particular those of the lined shore crab (*Pachygrapsus crassipes*, Randall 1840), were studied. Adult *P. crassipes* populations are widely distributed throughout southern California bays and coastal habitats, their spawning cycle is predictable, and larvae are easily sampled and identified (Ricketts et al. 1985, Schlotterbeck 1976). *Pachygrapsus crassipes* zoeae served as a model in this study for relatively strong swimming decapod larvae that potentially disperse through the coastal zone (e.g., Epifanio 1988, McConnaugha 1988). Specific goals of the proposed research were to: (1) develop and test the use of trace elements as tags to distinguish *P. crassipes* larvae originating from bay and open coast waters, (2) apply elemental fingerprinting to examine bay-

ocean exchange of *P. crassipes* larvae between San Diego Bay and nearby coastal waters, (3) examine the interaction of physical processes and larval behavior in determining the export and retention of larvae in San Diego Bay, and (4) study the effects of brood site and culture water on larval survivorship and development to establish whether there was any selective advantage for development in bay versus coastal waters.

The results of this research have been prepared as four papers for publication in the primary literature. Key findings are outlined below.

Vertical Migration Behavior and Horizontal Distribution of Brachyuran Larvae in a Low Inflow Estuary: Implications for Bay-Ocean Exchange

Tidally timed vertical migration has been well documented for brachyuran larvae that are preferentially exported or retained within partially mixed estuaries. However, the effectiveness of tidally timed vertical migration in an unstratified low inflow estuary (LIE), such as San Diego Bay, has not been addressed. This study examines the effect of larval vertical migratory behavior on dispersal by characterizing temporal and spatial (vertical and horizontal) distribution patterns of brachyuran larvae over tidal and diel cycles in SDB. The influence of temporal changes in larval vertical and horizontal distribution on larval retention and dispersal is evaluated. This research provides the first detailed description of the vertical and horizontal distribution of crab larvae within a recognized LIE and within any embayment along the West Coast of North America. Hydrodynamic modeling is combined with field surveys to examine hypotheses regarding dispersal in brachyuran larvae. A novel benthic sampling strategy was employed to establish the presence of larvae on the bottom of the bay during critical phases of the tide affecting transport between regions of SDB and between the bay and the nearshore coastal environment.

Net larval transport simulations, using a validated hydrodynamic model (TRIM2D) of San Diego Bay generated the prediction, *a priori*, that tidally timed vertical migration was necessary to transport larvae out of the bay, while larvae lacking vertical migratory behavior should be retained within SDB. Model simulations predicted that larvae with tidal rhythmic migration behavior experienced net transport towards the bay entrance (Fig. 1A-D). TRIM2D simulations of passive larvae, with vertically uniform distributions of zoeae, predicted that larvae would be retained within the back bay (Fig. 1E-H). This study examined hypotheses that brachyuran larvae sampled in SDB (1) migrate vertically into surface and bottom boundary layers causing enhanced export or retention from estuaries, and (2) are not homogeneously distributed horizontally in space between eastern, mid-channel and western stations along cross-channel transects in SDB. Brachyuran larvae from two families [i.e., *Pachygrapsus crassipes* (Grapsidae) and *Lophopanopeus* spp. (Panopeidae)] were studied at sites in central- and outer-SDB. Stage I *P. crassipes* zoeae exhibited tidally timed vertical migratory behavior (i.e., selective tidal stream transport) which enhanced their net export from the bay. *Pachygrapsus crassipes* zoeae were aggregated in surface layers (0 to 2 m) during nocturnal ebbing tides and exhibited behavior that effectively retarded transport back into the bay by exploiting zero velocity conditions associated with the benthic-boundary layer during flood tide (Table 1; Fig. 2). *Lophopanopeus* spp. exhibited no clear tidally timed rhythmic migration or aggregation patterns. All developmental stages were collected within SDB, indicating larvae were retained

within SDB throughout meroplanktonic development (Table 1; Fig. 2). Heterogeneous horizontal distributions of stage I *P. crassipes* and *Lophopanopeus* spp. larvae reflect hatching sites and physical circulation features which concentrate larvae at specific stations. Differences in larval behavior of the sort observed for grapsid and panopeid larvae in SDB may ultimately affect the success of recruitment to adult populations.

The vast majority of studies considering temporal and spatial distributions of brachyuran larvae have been conducted in estuaries with significant freshwater input (e.g., partially mixed estuaries) resulting in non-tidal residual flows and net downstream transport and flushing of passive and uniformly distributed particles. The present study considered the vertical migratory behavior of crab larvae in SDB, a low inflow estuary which experiences negligible or no net downstream flow due to limited freshwater input. Swimming behavior mediated retentive mechanisms were not necessary for retention of *Lophopanopeus* spp. larvae within SDB. Panopeid species studied in partially mixed estuaries tend to exhibit tidally timed vertical migrations which maintain their position within estuaries (Cronin & Forward 1979, 1982; Lambert & Epifanio 1982; Johnson 1985). Stage I *P. crassipes* zoeae employed selective tidal stream transport that facilitated their export from SDB to the coastal ocean. Tidal stream transport appears necessary for *P. crassipes* larvae to exit SDB, based on the observation of retention of panopeid larvae (which lack vertical migration) within SDB through all planktonic stages of development. The efficiency of tidally timed vertical migration in promoting export appears to be enhanced by *P. crassipes*' ability to exploit zero velocity at the sediment-water interface during flood tides.

Development and Application of Elemental Fingerprinting to Track the Dispersal of Marine Invertebrate Larvae

The early life history of many marine benthic invertebrate and fish species involves a planktonic larval stage that allows exchange of individuals among separated adult populations. Inability to determine sources of planktonic larvae and to consequently track their fate *in situ* has limited our understanding of dispersal and recruitment mechanisms. The purpose of this study was to develop and test the use of naturally-occurring trace elements and pollutants as viable tags to distinguish origins of planktonic larvae and to use this method to examine bay-ocean exchange processes in the striped shore crab, *Pachygrapsus crassipes* (Grapsidae, Randall 1840). This research has: (1) described an analytical protocol to measure trace element concentrations in individual crab larvae, (2) characterized the elemental composition of individual *P. crassipes* larvae sampled from embayments and exposed coastal habitats of southern California, (3) tested the discriminatory power of observed elemental differences as a tag to determine larval origin, and (4) applied this approach to examine the exchange of stage I zoeae between San Diego Bay (SDB) and nearshore coastal habitats. Based on observations of tidally associated vertical migration of larvae, we initially hypothesized that transport of stage I zoeae originating inside SDB should be unidirectional and out of the Bay.

Trace elements are effective site markers for estuaries because run-off and pollutant loading often impart distinct elemental signatures to bay habitats relative to nearshore coastal environments. Crab larvae originating from San Diego Bay (SDB) were distinguished from those originating in neighboring embayments and exposed coastal habitats by comparing multiple trace

element concentrations ('fingerprints') in individuals (Fig. 3, Table 2). Discriminant function analysis (DFA) was used to characterize stage I zoeae of the striped shore crab, *Pachygrapsus crassipes*, of known origin (reference larvae) via trace elemental composition (i.e., Cu, Zn, Mn, Sr, Ca) (Fig. 4). A validation of the classification algorithms indicated that 93 percent of SDB larvae (n=39 larvae) and 96 percent of non-SDB larvae (n=57 larvae) were correctly identified (Table 3). Linear discriminant functions were used to identify the origin and characterize the flux of stage I *P. crassipes* zoeae between SDB and the nearshore coastal environment during one spring tidal cycle. Elemental fingerprinting revealed that most (87%) of the stage I larvae collected at the bay entrance during the flood tide were larvae of SDB origin that were reentering the bay. Nearly one third of zoeae sampled (32%) at the entrance during ebb tide were coastal larvae leaving the bay and returning to open water. These results contrast with the unidirectional transport of zoeae out of the bay predicted from stage I vertical migrations. Because zoeal survivorship differs in SDB and coastal waters, the bi-directional bay-ocean exchange shown here has significant implications for the dynamics of *P. crassipes* populations. Trace-elemental fingerprinting of invertebrate larvae promises to facilitate investigations of many previously intractable questions about larval transport and dynamics of nearshore species.

Use of Elemental Fingerprinting to Assess Net Flux and Exchange of Brachyuran Larvae Between Regions of San Diego Bay, California and Nearshore Coastal Habitats

Marine benthic invertebrate populations found in estuarine or coastal habitats often exchange larvae. However, the dynamics of larval exchange are poorly understood because of difficulties in (1) making synoptic assessments of horizontal and vertical larval distribution patterns over large areas for extended periods of time and (2) determining the origins of field-sampled larvae. This study examines how temporal changes in the vertical and horizontal distribution of crab larvae (i.e., *Pachygrapsus crassipes* and *Lophopanopeus* spp.) affect larval transport. Larval concentration estimates were combined with concurrently sampled water velocity measurements to test the hypothesis that heterogeneous vertical and horizontal distributions of brachyuran larvae affect larval flux between regions of SDB and between SDB and nearshore coastal waters. Elemental fingerprinting was employed to analyze a subset of stage I *P. crassipes* larvae sampled in middle and outer regions of SDB to test the null hypothesis that all zoeae sampled within the bay originated from inside SDB.

First order larval flux approximations between inner and outer regions of SDB and between the bay and nearshore coastal habitats corroborate transport inferred from larval behavior. The net flux of stage I *P. crassipes* zoeae integrated in this study over a semi-diurnal tide was estimated to be from inner to outer regions of SDB at the mid bay (CBB) sampling site (3.65×10^6 ind) and from outer SDB to nearshore coastal waters at the entrance (SDBE) sampling site (8.63×10^8 ind) (Table 4). The net flux for combined stage I and post-stage I *Lophopanopeus* spp. larvae sampled at the CBB site was from the outer to inner regions of SDB (1.28×10^6 ind; Table 4), suggesting retention within the bay through larval development. Trace elemental fingerprinting of stage I *P. crassipes* zoeae revealed bi-directional exchange between SDB and the nearshore coastal environment when unidirectional transport out of SDB was predicted from swimming behavior. Approximately 4.3% of stage I *P. crassipes* zoeae sampled in the mid region of SDB originated from outside SDB, while 26.4% of zoeae sampled at the

entrance originated from outside SDB (Fig. 5). Combined use of trace elemental fingerprinting and synoptic field sampling techniques will facilitate understanding of larval transport and ultimately population dynamics of nearshore species.

A broader objective of this study was to develop a methodology to evaluate net larval transport using state of the art analytical techniques for determining temporal and spatial variations in water velocity flow fields, larval distribution and larval origins. The effect of temporal changes in the vertical distribution of decapod larvae on net transport has been well documented, especially for brachyurans found within estuaries. This study revealed that the horizontal distribution of larvae also has a significant effect on net larval transport estimates. In addition to the temporal-spatial distribution of larvae, the distribution of adult source and hydrodynamic processes of SDB had an effect on net transport and bay-ocean exchange of larvae. Knowledge of *P. crassipes* zoeal origins allowed more accurate flux measurements to be made and provided insights into potential exchange of larvae between bay and coastal populations. The ability to accurately assess larval flux rates and to discriminate larval origins offers the potential for improved understanding of many aspects of larval dynamics in estuarine and nearshore ecosystems. Larval exchange between bay and coastal populations, and among populations within bays, may greatly influence larval survivorship, gene flow and the ability of populations to be self-sustaining.

Development and Survival of Striped Shore Crab Larvae (*Pachygrapsus crassipes*) in Bay vs Coastal Habitats

The striped shore crab, *Pachygrapsus crassipes*, lives in both protected embayments and exposed nearshore coastal habitats and larvae may develop in either setting. This study compared the survivorship and development of *P. crassipes* zoeae brooded and cultured in two southern California embayments (San Diego Bay, Mission Bay) and a nearby exposed coastal habitat (Dike Rock). Newly released larvae originating from 3 sites were reared in the laboratory in waters from each site through to the megalopal stage. The null hypothesis was that there are no differences in the development rate or survival of zoeae brooded in or developing in waters of southern Californian embayments (San Diego Bay [SDB] Mission Bay [MB]) when compared with zoeae developing in a nearby exposed coastal environment (Dike Rock [DR]).

Larval cultures maintained in seawater collected from the open coast experienced higher survivorship during zoeal development, exhibited a higher percentage of stage VI zoeae surviving to the post-larval megalopal stage, and yielded a larger percentage of viable megalopae than larvae reared in seawater collected from either San Diego Bay or Mission Bay (Fig. 6,7). Culture water and brood site did not affect zoeal development time. Larvae brooded in Mission Bay were more likely to survive zoeal stages of development than larvae brooded in San Diego Bay or Dike Rock. Megalopae which were brooded in Dike Rock and San Diego Bay habitats had greater megalopal survival times than those originating from Mission Bay. Brood site had no effect on the percentage of zoeae surviving to the megalopal stage of development, zoeal development time, or molting success of stage VI zoeae to the megalopal stage of development. Thus, brood site and culture water source will influence *P. crassipes*' rate of development and survivorship, and as a consequence, the source of individuals ultimately recruiting to adult populations.

Conclusions and comments

Many marine invertebrate organisms have a planktonic larval stage of development that promotes the exchange of individuals between isolated adult populations inhabiting different regions of a bay, different bays, or bay and coastal habitats. The duration of the planktonic stage of development as well as larval behavior enhance or retard larval dispersal. This has been particularly well documented for brachyuran larvae (e.g., Epifanio 1988, McConnaugha 1988). The present study employed larval pump sampling, concurrent characterization of hydrographic features (i.e., tidal currents), hydrodynamic simulations, and a novel larval fingerprinting technique to study brachyuran larval exchange between San Diego Bay and exposed coastal habitats. *Pachygrapsus crassipes* larvae (Decapoda, Grapsidae) were selected for study of bay-ocean exchange processes since adult populations inhabit protected embayments as well as exposed coastal rocky intertidal habitats.

The majority stage I *P. crassipes* zoeae sampled in SDB exhibited tidally timed, vertical migratory behavior (i.e., selective tidal stream transport) that enhanced their net transport from the bay (Chapter II). Larvae aggregated in surface layers during nocturnal ebbing tides and retarded transport back into the bay by exploiting reduced velocity conditions in the bottom boundary layer during flood tides. Post-stage I *P. crassipes* zoeal stages of development were rarely sampled within SDB. The lack of post-stage I zoeae within SDB agrees with results from flux studies which revealed net transport of stage I larvae from inner to outer regions of SDB and ultimately into nearshore coastal waters (Chapter IV). Larval behavior and flux estimates suggest that most *P. crassipes* larvae spawned within embayments exit while still in the initial larval stage of development. Zoeal development is likely completed in coastal waters until molting into the post-larval stage of development (i.e., megalop), which actively recruit to adult habitats located within embayments and on exposed coastal shores.

The majority of studies on brachyuran larval migratory behavior reveal some larvae that either do not migrate in phase with the majority or do not migrate at all (e.g., Queiroga et al. 1997, Garrison et al. 1999). The non-tidal, residual flow characteristic of partially mixed estuaries, where most larval studies have been conducted, results in net downstream transport and flushing of non-migratory larvae. This is not the case for SDB, a low inflow estuary (LIE) where freshwater input is negligible or absent for extended periods of time (Largier et al. 1996, 1997). As a result of limited freshwater input, LIE's are characterized by sluggish circulation and extended residence times for tidally sheltered waters and passive constituents, including non-migratory larvae. For example, *Lophopanopeus* spp. zoeae, sampled concurrently with *P. crassipes* larvae during this study, did not exhibit rhythmic migratory behavior and were retained within the bay through larval development. Those *P. crassipes* larvae lacking rhythmic migrations should also be retained within the bay.

The retention of some *P. crassipes* larvae and most *Lophopanopeus* spp. larvae within SDB and their potential for completing development within the Bay has important implications for population sustainability within SDB and in other LIE's. Despite experimental evidence which suggests that *P. crassipes* larvae reared in SDB seawater experience slower development and reduced survivorship than larvae reared in coastal water, the retention and subsequent recruitment of SDB spawned zoeae to adult SDB populations (i.e., self-seeding) could represent

a major source of recruitment. Retention within embayments during planktonic development would limit dispersal and increase the probability that post-larval recruits would locate suitable adult habitat (e.g., Cronin and Forward 1982, Lambert and Epifanio 1982, Epifanio 1988).

In southern California, many physical oceanographic features have been recognized which can enhance larval transport away from nearshore coastal waters, effectively eliminating their return and recruitment to coastal populations. These mechanisms include offshore transport associated with upwelling-induced equatorward coastal jets, eddy structures, and filaments associated with the California current (Haury et al 1986, Roughgarden et al. 1984, Roughgarden et al. 1988, Farrell et al. 1991, Strub et al. 1991, Wing et al. 1998). By splitting larval development between bay and coastal habitats, *P. crassipes* effectively hedges its bets for successful recruitment, especially during years when recruitment by larvae developing in nearshore coastal waters is low.

Self-seeding is not likely from SDB spawned larvae which exit the bay and develop in coastal waters. Average summer surface currents recorded off southern California can be extremely rapid (6.1 cm sec^{-1}) (Winant and Bratkovich 1981), and larvae exported from SDB are likely to be transported a considerable distance from the bay entrance before they are ready to settle. Levin (1983) suggested that longshore currents off southern California could carry polychaete larvae with a two-week planktonic phase 80 to 120 km from Mission Bay in southern California. Dispersal potential is likely to be even greater during the 6 to 8 week duration of *P. crassipes* zoeal development in the absence of behavioral (i.e., swimming) or physical retentive features (i.e., eddies, fronts).

The relative importance of self-seeding versus recruitment by larvae originating from neighboring embayments or coastal populations in sustaining adult *P. crassipes* populations within SDB is unknown. Southern California has lost up to 95 % of its wetlands, the predominant natural habitat for adult *P. crassipes* within embayments (Hiatt 1948). The loss of wetland habitat precludes establishing the historical significance of self-seeding. However, understanding the importance of bay versus coastal spawned larvae in maintaining populations in both habitats is critical if current populations are to be effectively managed and preserved. This understanding will only be possible when larval tracking techniques currently under development (e.g., trace elemental fingerprinting, genetic tags) allow the origin of newly recruited individuals to be determined.

Trace elemental fingerprinting determined that stage I *P. crassipes* zoeae originating from populations outside SDB were present inside the bay. Previous studies have combined information on spawning sites, larval behavior, and hydrographic features to infer potential larval dispersal trajectories. This study exemplifies what can be learned by determining the origin and tracking the dispersal of individual larvae. The ability to determine the origin of field sampled larvae allowed the observation of bi-directional exchange of stage I *P. crassipes* larvae between SDB and nearshore coastal waters, even when net transport was from bay to coastal habitats. Calculations involving known larval concentrations and an estimated instantaneous mortality rate for *P. crassipes* zoeae, suggest that approximately 150 non-SDB spawned *P. crassipes* zoeae would be expected to complete larval development from an estimated 5.9×10^7 stage I zoeae in the vicinity of the CBB sampling site, and serve as potential recruits to adult habitats.

There are many questions that remain to be answered regarding the transport and dispersal of *P. crassipes* zoeae, and meroplankton in general. The fate of subsequent larval stages of *P. crassipes* development could not be addressed in the current study because a limited numbers of post-stage I zoeae were sampled with the larval pump. Later stages, especially the post-larval megalopal stage of development, will be critical to a better understanding of recruitment dynamics. Study of larval stages may allow us to identify preferred habitats for larval development prior to recruitment inside and outside SDB. The trace elemental fingerprinting technique developed in this study did not effectively distinguish larvae that originated from neighboring embayments and coastal populations. More sensitive analytical protocols and instrumentation capable of more sensitive analyses may improve methodologies established here.

Ultimately, it is hoped that this research has provided a novel approach that can be improved and applied in future studies to assess the relative significance of self-seeding and larval exchange between bay and coastal populations as alternate recruitment mechanisms. The relation between larval exchange and long-term persistence of coastal and bay populations will have important implications for the way that coastal habitats are managed in the future. A viable technique for assessing the degree of self-seeding of estuarine populations will aid future management of remaining coastal habitats, especially for fragmented systems such as mudflats and salt marshes of southern California (Fairweather 1991). San Diego Bay, like many other urbanized bays, has lost much of its natural habitat to industrial, commercial and military development. The interdependence of estuarine and coastal populations is highlighted when there are potential consequences of losing one habitat type (e.g., wetlands) on another (e.g., rocky shores).

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Table 1. One-way analysis of variance comparing larval abundance estimates for surface (0-2 m = T), middle-depth (5-7 m = M), and bottom (<2 mab, meters above the bottom = B) layers sampled during ebbing and flooding tides during July, August and September 1997 sampling periods. Stage 1 zoeae were analyzed for *Pachygrapsus crassipes*; stage I and post-stage I *Lophopanopeus* spp. zoeae were combined for the analysis presented, however separate analyses of stage I and post-stage I larvae also failed to identify any statistical differences. Statistically significant multiple comparison results (Bonferroni corrected *Student-t*) are given for each Anova which identified significant differences. The July 1997 sampling period was not analyzed since the sediment-water interface was not adequately sampled for *P. crassipes* during this sampling period.

Sampling Period	Tidal Phase	n	F-ratio	p-value	<i>Student-t</i>
<i>P. crassipes</i>					
4-5 August 1997	Ebb	12	4.367	0.038	T > M, B
	Flood	30	3.091	0.060	-
18-19 August 1997	Ebb	33	4.010	0.028	T, M > B
	Flood	30	7.453	0.002	T, M < B
1-2 September 1997	Ebb	33	5.694	0.008	T > M, B
	Flood	51	12.787	<0.001	T, M < B
<i>Lophopanopeus</i> spp.					
21-22 July 1997	Ebb	33	1.207	0.312	-
	Flood	42	0.978	0.384	-
1-2 September 1997	Ebb	33	1.573	0.223	-
	Flood	51	0.759	0.473	-

Table 2. Mean (± 1 SD) elemental concentrations ($\mu\text{g}\cdot\text{kg}^{-1}$) of stage I *Pachygrapsus crassipes* reference zoaea sampled from inner and outer regions of San Diego Bay (SDB), neighboring embayments and coastal sites (see Fig. 1). One factor analysis of variance (ANOVAs) was used to determine significant differences among sites. Concentrations for laboratory hatched stage I *P. crassipes* zoaea are also presented. All mean estimates are based on $n=96$ individuals for reference zoaea and $n=6$ to 12 individuals for laboratory hatched zoaea. A posteriori multiple comparison results are given in Figure 2.

Element	Reference Zoaea				Laboratory Hatched Zoaea		
	Inner SDB	Outer SDB	Neighboring Embayments	Coastal Sites	ANOVA		Inner SDB
					$F_{3,92}$	p	
Aluminum	34.1 (23.6)	22.3 (7.5)	12.3 (10.6)	8.9 (5.7)	17.34	$p<0.001$	24.2 (10.8)
Copper	23.5 (29.1)	6.0 (8.8)	1.7 (2.2)	1.0 (0.8)	12.51	$p<0.001$	33.1 (22.6)
Zinc	25.8 (17.7)	18.1 (13.0)	7.3 (6.6)	3.4 (2.6)	21.53	$p<0.001$	-
Strontium	132.1 (82.2)	75.9 (77.3)	99.1 (57.2)	141.5 (103.1)	3.12	$p=0.030$	94.3 (22.7)
Manganese	10.4 (8.2)	8.5 (7.3)	1.2 (0.9)	1.0 (1.0)	22.33	$p<0.001$	-
Magnesium	1700.9 (1203.0)	2057.6 (1087.1)	3632.0 (3345)	5699.3 (7198.6)	3.96	$p=0.011$	1131.4 (531.5)
Calcium	11011.5 (5453.6)	13016.2 (5076.1)	8706.5 (5625.7)	10690.2 (5342.8)	2.21	$p=0.092$	8695.8 (2139.1)
							13741.8 (1399.2)

Table 3. Validation results of the classification algorithm. (a) Classification matrix indicating the predicted origin for larvae comprising the model data set and (b) Cross-validation using a 'jackknifed' classification matrix (described in methods) to test the robustness of the classification algorithm.

(a) Classification Matrix

True Identity	Predicted Identity		% Correct
	SDB	not SDB	
SDB	37	3	93
not SDB	2	54	96
Total	39	57	95

(b) Jackknifed Classification Matrix

True Identity	Predicted Identity		% Correct
	SDB	not SDB	
SDB	36	4	90
not SDB	3	53	95
Total	39	57	92

Table 4. Larval flux estimates (no. ind.) between (A) inner and outer regions of San Diego Bay (SDB) for stage I *Pachygrapsus crassipes* and combined stages of *Lophopanopeus* spp. zoeae and (B) between SDB and nearshore coastal waters for stage I *P. crassipes* zoeae. Flux measurements were made over a complete semi-diurnal tidal cycle (12.4 hr) at the Coronado Bay Bridge (CBB; 21-22 July 1997) and San Diego Bay Entrance (SDBE; 18-19 August 1997) sites (see Fig. 1). A net larval flux estimate and flux estimates for each depth (surface, mid-depth, and bottom layers) and horizontal station (eastern, mid-channel, western stations) (see Fig. 2) are provided. Negative values indicate net flux from inner to outer regions of SDB at the CBB site and out of SDB at the SDBE site. The sediment-water interface was not sampled during the 21-22 July 1997 sampling period (bottom, 0.5-2 mab, meters above the bottom). Sectional flux estimates are provided for ebb and flood tidal phases. Net flux estimates are provided for the complete semi-diurnal tide.

Sample Site	Sample Depth	Ebb Tidal phase			Flood Tidal Phase		
		Western	Mid-Channel	Eastern	Western	Mid-Channel	Eastern
A. CBB <i>P. crassipes</i>	Surface	-3.0×10^5	-1.2×10^6	-2.6×10^6	1.4×10^5	4.6×10^5	6.6×10^5
	Mid-depth	-4.8×10^5	-2.1×10^5	-1.1×10^6	8.4×10^4	2.5×10^5	6.3×10^5
	Bottom	-3.3×10^5	-4.4×10^5	-1.0×10^5	2.7×10^5	9.1×10^4	4.4×10^5
	Flux			-6.7×10^6			3.0×10^6
	Net Flux						-3.65×10^6
<i>Lophopanopeus</i> spp.	Surface	-2.5×10^6	-6.0×10^6	-6.9×10^6	8.9×10^6	4.0×10^6	1.8×10^6
	Mid-depth	-2.2×10^6	-3.6×10^6	-2.9×10^6	2.1×10^6	5.4×10^6	4.6×10^6
	Bottom	-2.6×10^6	-5.5×10^6	-3.2×10^6	2.4×10^6	4.7×10^6	2.8×10^6
	Flux			-3.5×10^7			3.7×10^7
	Net Flux						1.28×10^6
B. SDBE <i>P. crassipes</i>	Surface	-7.2×10^7	-2.4×10^8	-3.1×10^8	7.3×10^6	3.3×10^6	1.1×10^6
	Mid-depth	-3.6×10^7	-1.8×10^8	-9.2×10^7	3.6×10^7	4.7×10^6	7.2×10^5
	Bottom	-6.4×10^5	-3.5×10^5	-1.4×10^5	3.6×10^6	4.8×10^6	2.0×10^5
	Flux			-9.2×10^8			6.2×10^7
	Net Flux						-8.63×10^8

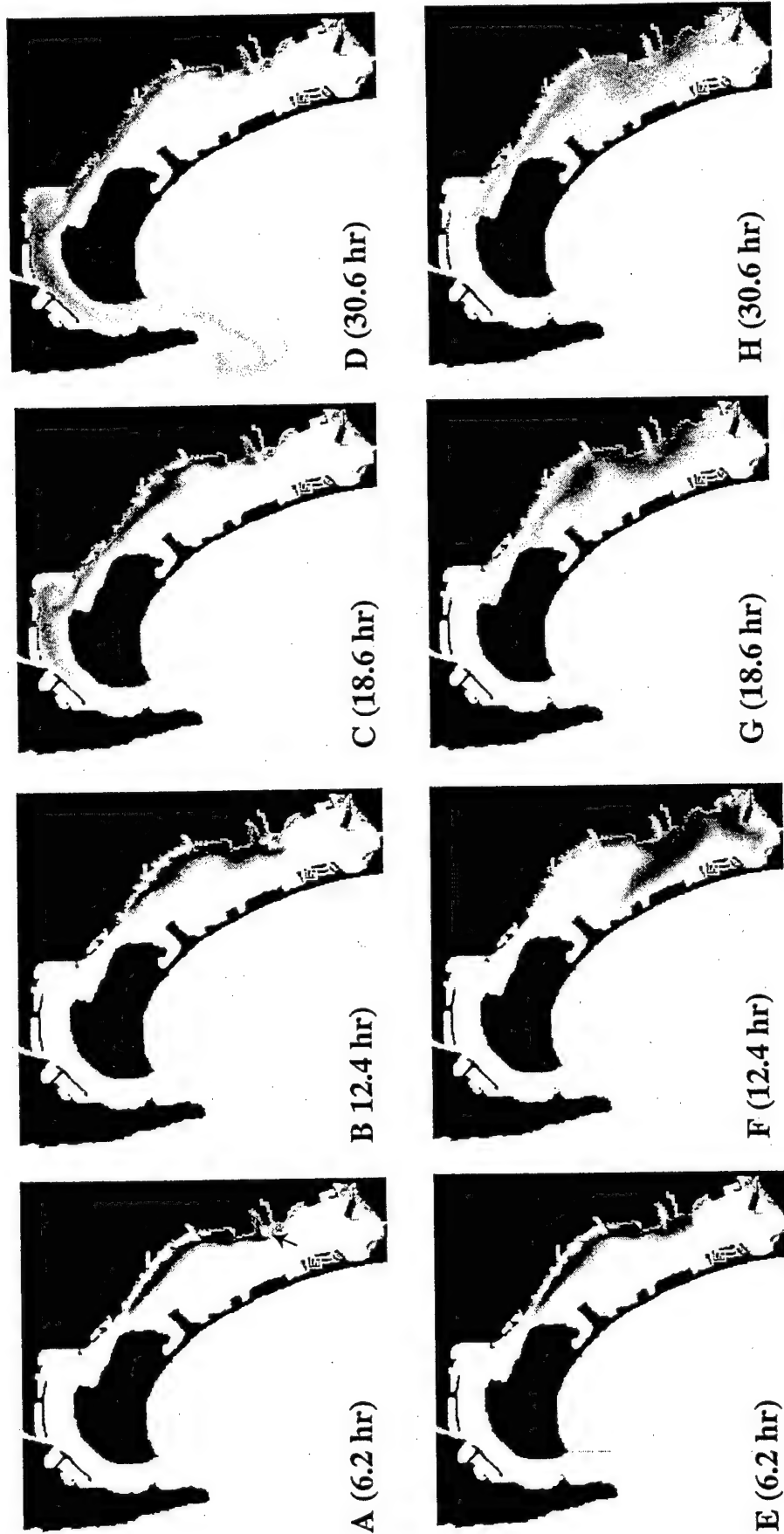


Figure 1. Tidal Residual Intertidal Mudflat Model (TRIM2D) simulations for brachyuran larvae released from identified intertidal and high intertidal habitats on the eastern side of SDB. Simulations incorporate vertical migratory behavior observed in stage I *Pachygrapsus crassipes* zoeae, which aggregate in surface layers during flood tide and in benthic layers during ebb tide (panels A-D). The simulated larvae are concentrated in the eastern half of the channel at the CBB site and experience a net transport towards the SDB entrance (A-D). Simulations without tidally timed vertical migrations (e.g., as in *Lophopanopeus* spp.) produce larval distributions concentrated in back of SDB (panels E-F). Grey scale shows concentration gradients, with darker areas indicating higher concentrations, following the initial ebb tide (6.2 hours post-release; panels A, E), flood tide (12.4 hr post-release; panels B, F), ebb tide (18.6 hr post-release; panels C, G), and flood tide (30.6 hr post-release; panels D, H). The general release site for simulated larval release was Sweetwater Marsh (see arrow in panel A).

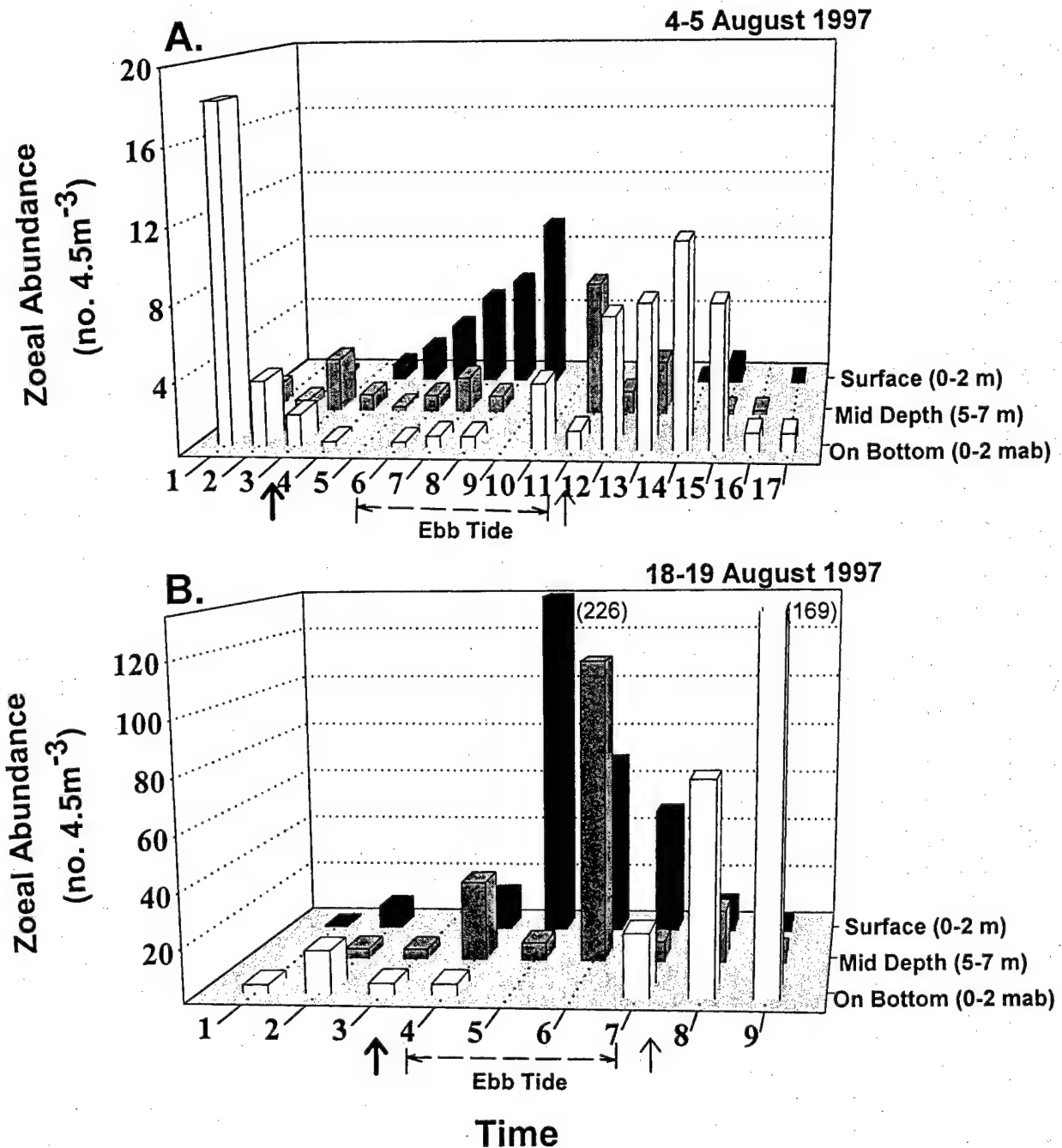


Figure 2. *Pachygrapsus crassipes*. Temporal and vertical distribution of stage I zoeae sampled at the San Diego Bay Entrance (SDBE) transect on (A) 4-5 August 1997 and (B) 18-19 August 1997. Zoeal abundance for each transect was summed across eastern, middle and western stations for each depth, respectively. The dashed line on the x-axes indicates ebb tide. The thick and thin vertical arrows on the x-axes indicate approximate times of sunset and sunrise, respectively.

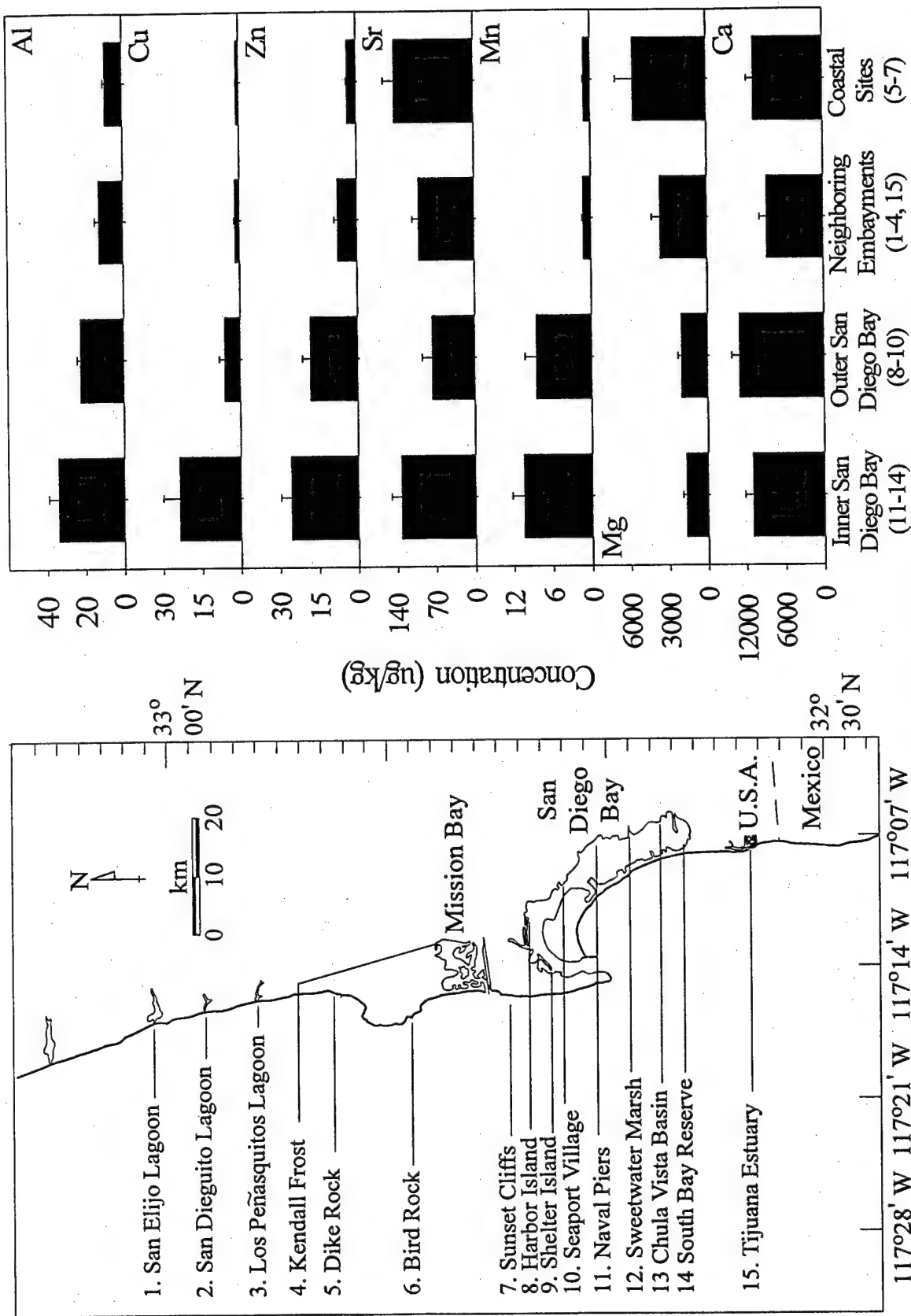


Figure 3. Trace element gradients in stage I zoeal crabs, *Pachygrapsus crassipes*. Mean trace element concentration (\pm 1SE) per individual larva (mg kg⁻¹) sampled from inner and outer regions of San Diego Bay, neighboring embayments and exposed coastal sites. Bars which share a letter are not statistically different at the Bonferroni corrected significance level ($p=0.017$) for a posteriori multiple comparisons. Sampling sites pooled within each region are indicated by numbers in parentheses that correspond to numbered sites on the chart.

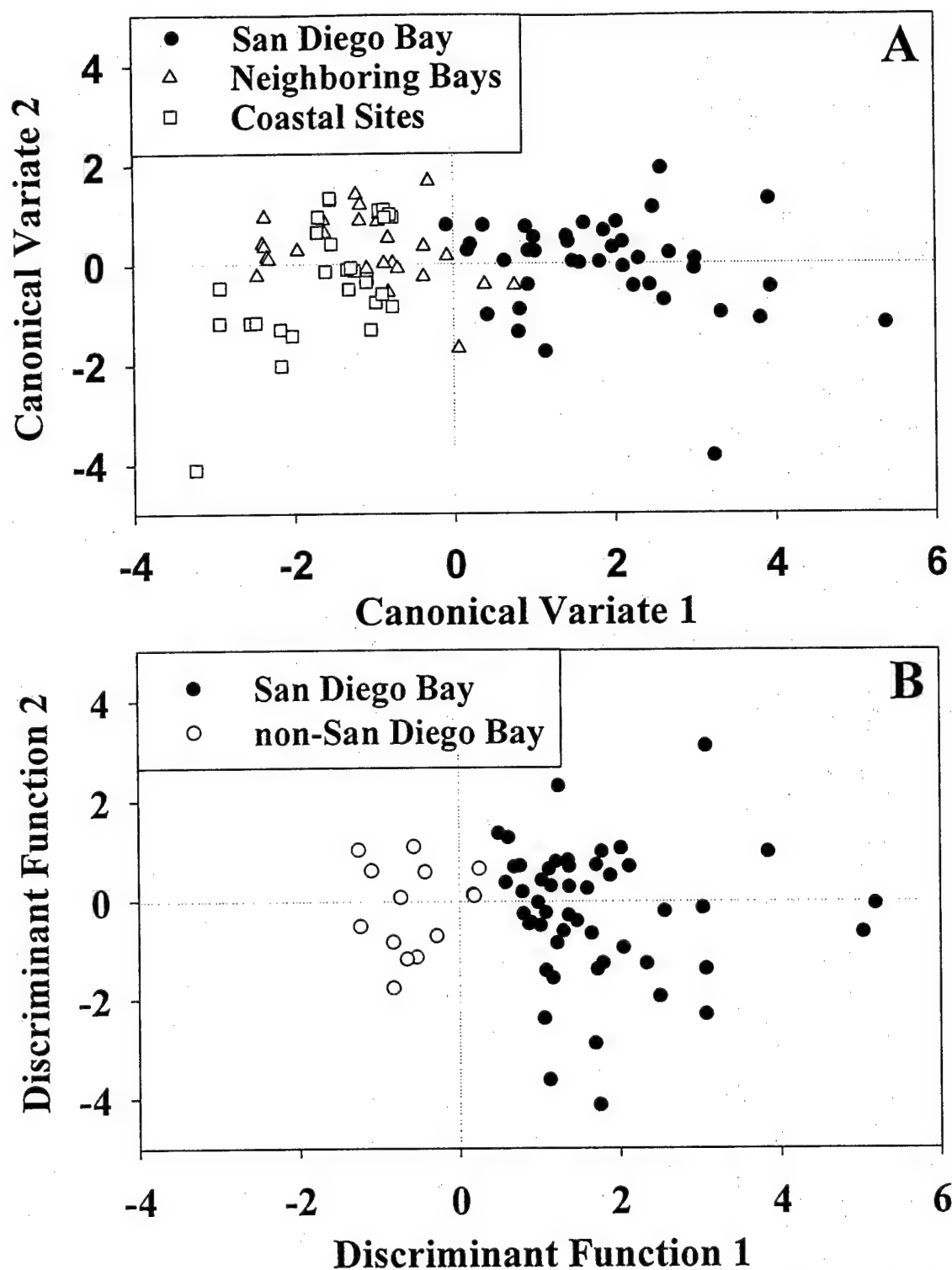


Figure 4. Trace elemental fingerprints characteristic of stage I *Pachygrapsus crassipes* zoea sampled from San Diego Bay, neighboring embayments and coastal sites. (A) First and second canonical variable scores correspond to individual zoea of known origin. The first canonical variable (Canonical Variate 1) accounts for 98% of the dispersion between groups. (B) Plot of two discriminant functions for zoea of unknown origin sampled at the entrance of San Diego Bay over a tidal cycle. Note that the points cluster within San Diego Bay and non-San Diego Bay groupings defined by discriminant function analysis shown in the upper panel.

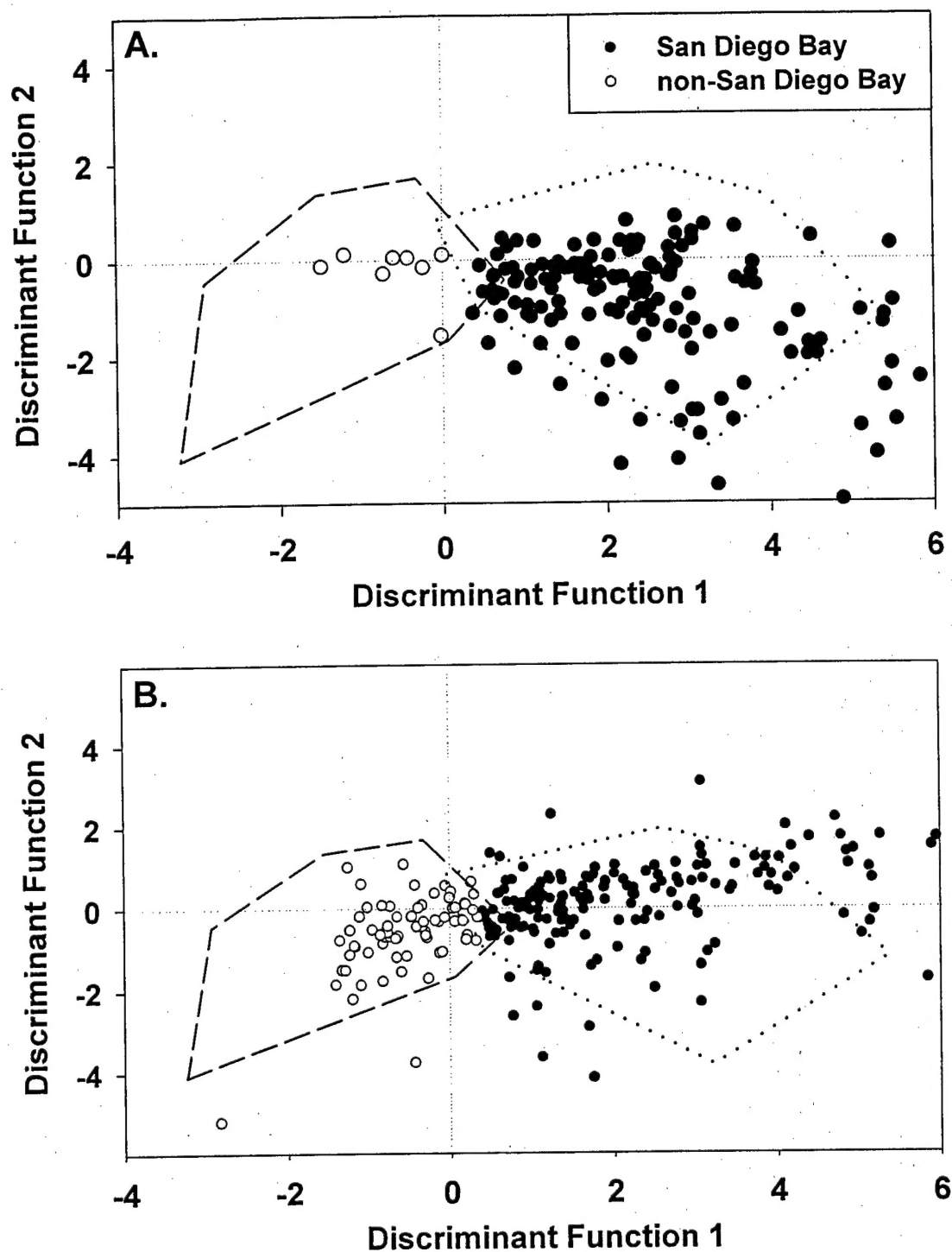


Figure 5. Plot of discriminant function values 1 and 2 (based on trace elemental composition) for zoeae of unknown origin sampled over a tidal cycle at the (A) Coronado Bay Bridge and (B) San Diego Bay Entrance sampling sites. Discriminant function space defined from reference stage I *Pachygrapsus crassipes* zoeae (of known origin) are shown for larvae from San Diego Bay (dotted line) and non-SDB (i.e., neighboring embayments and coastal sites; dashed line) sampling sites. Note that most of the points, each of which represents a single larva of unknown origin, cluster within discriminant function space defined by the dotted and dashed lines, with only limited overlap.

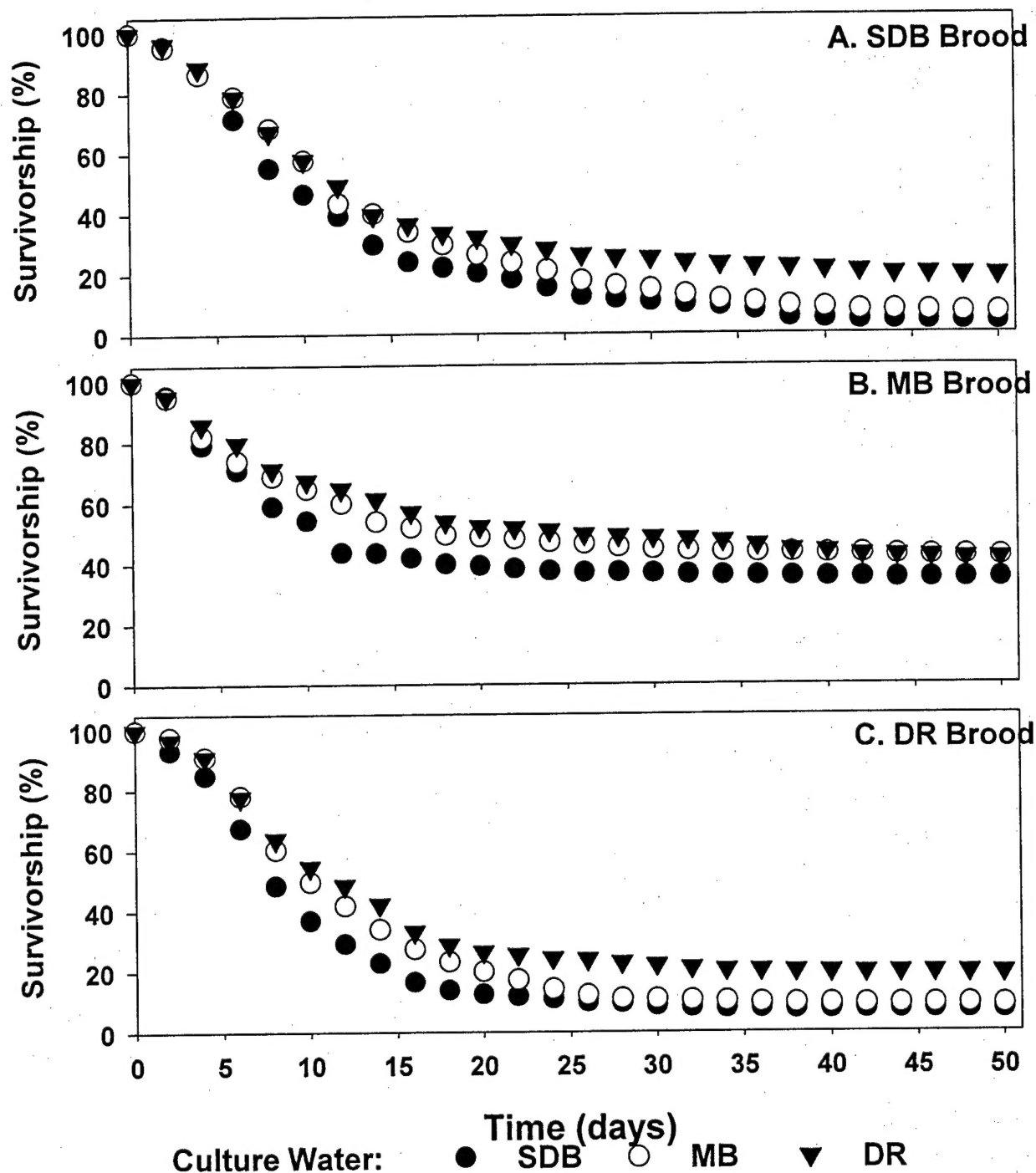


Figure 6. Culture water effects on laboratory cultured crab larvae, *Pachygrapsus crassipes*, expressed as percent survivorship. Mean survivorship estimates within each plot (A-C) are independent of larval origin since larvae were brooded in (A) Sweetwater Marsh, San Diego Bay (SDB), CA, (B) Northern Wildlife Preserve, Mission Bay (MB), CA, or (C) Dike Rock (DR), La Jolla, CA. All curves are terminated at day 50, but cultures lasted an average of 98 ± 7 d (SD). Error bars are not shown for the sake of clarity. Statistical comparisons among sites are given in the text.

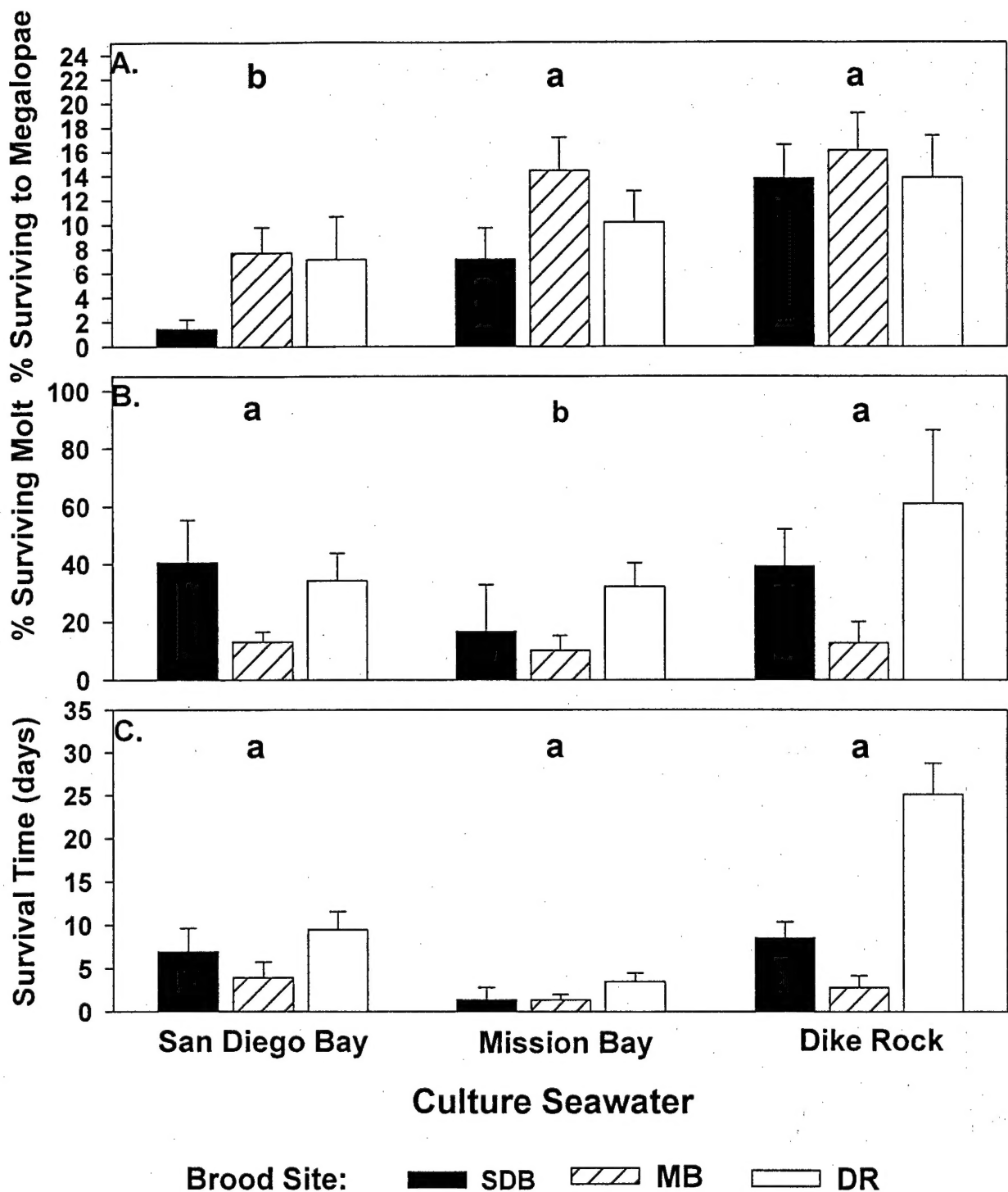


Figure 7. Culture water and brood site effects on (A) the mean percentage of stage I zoea, *Pachygrapsus crassipes*, surviving to the megalopal stage of development, (B) the mean percentage of stage VI zoea, *Pachygrapsus crassipes*, which attempted and survived molting from the sixth zoeal stage of development to the megalopal stage, and (C) the survival time (days) for laboratory cultured crab megalopae, *Pachygrapsus crassipes*, since molting from the sixth zoeal stage of development. Error bars indicate ± 1 SE. MB= Mission Bay, SDB= San Diego Bay, DR= Dike Rock. Culture water treatments sharing the same letter (a, b) are not significantly different. Brood site results are given in the text.

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13. ABSTRACT (Maximum 200 words) Further advancement in understanding the dynamics of larval exchange between estuarine and coastal marine benthic invertebrate populations requires (1) knowing the origins of field-sampled larvae and (2) synoptic assessments of horizontal and vertical larval distribution patterns over large areas for extended periods. Larval concentration and water velocity data were sampled concurrently and used to estimate larval flux rates between regions of San Diego Bay (SDB) and nearshore coastal waters in southern California. Simulations with a 2-D hydrodynamic model of SDB indicated widely differing larval transport probabilities depending on whether tidal vertical migration behavior occurs in the water column. Field studies indicate that crab (<i>Pachygrapsus crassipes</i>) zoeae migrate vertically in SDB, a behavior that promotes transport out of the Bay. In contrast, larvae of other crab species (<i>Lophopanopeus spp.</i>), which do not migrate vertically, are retained within SDB during development. An elemental larval fingerprinting technique (based on Cu, Zn, Al, Mn, and Sr) was developed to distinguish SDB from non-SDB spawned <i>P. crassipes</i> zoeae. With this method, bi-directional larval exchange was observed between SDB and coastal waters. Approximately 26% of <i>P. crassipes</i> larvae observed at the SDB entrance, and 5% at a mid-bay site, originated outside the Bay. This exchange is likely to have significant consequences for larval populations. Laboratory experiments revealed reduced survivorship in larvae spawned from or reared in SDB water relative to pristine coastal waters. Combined use of trace elemental fingerprinting and synoptic field methods can facilitate understanding of larval transport and ultimately population dynamics of coastal species.				
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